Clinical, biological, and molecular characteristics of clonal mast cell disorders presenting with systemic mast cell activation symptoms

Iván Álvarez-Twose, MD,[a,m]* David González de Olano, MD,[b,m]* Laura Sánchez-Muñoz, MD, PhD,[a,m] Almudena Matito, MD,[a,m] Maria I. Esteban-López, MD,[c] Arantza Vega, MD, PhD,[d] Maria Belén Mateo, MD,[e] Maria D. Alonso Díaz de Durana, MD,[f] Belén de la Hoz, MD, PhD,[g,m] Maria D. del Pozo Gil, MD, PhD,[h] Teresa Caballero, MD,[i] Ana Rosado, MD,[f] Isabel Sánchez Matas, MD,[j] Cristina Teodósio, MSc,[k,m] María Jara-Acevedo, Msc,[k,m] Manuela Mollejo, MD, PhD,[l] Andrés García-Montero, PhD,[k,m] Alberto Orfao, MD, PhD,[k,m]† and Luis Escribano, MD, PhD[a,m]† *Toledo, Madrid, Segovia, Guadalajara, Ciudad Real, Logroño, and Salamanca, Spain*

Background: Systemic mast cell activation disorders (MCADs) are characterized by severe and systemic mast cell (MC) mediators–related symptoms frequently associated with increased serum baseline tryptase (sBt).

Objective: To analyze the clinical, biological, and molecular characteristics of adult patients presenting with systemic MC activation symptoms/anaphylaxis in the absence of skin mastocytosis who showed clonal (c) versus nonclonal (nc) MCs and to provide indication criteria for bone marrow (BM) studies.

Methods: Eighty-three patients were studied. Patients showing clonal BM MCs were grouped into indolent systemic mastocytosis without skin lesions ($ISMs^-$; n = 48) and other c-MCADs (n = 3)—both with $CD25^{++}$ BM MCs and either positive mast/stem cell growth factor receptor gene (*KIT*) mutation or clonal human androgen receptor assay (HUMARA) tests—and nc-MCAD (CD25-negative BM MCs in the absence of *KIT* mutation; n = 32) and compared for their clinical, biological, and molecular characteristics.

Results: Most clonal patients (48/51; 94%) met the World Health Organization criteria for systemic mastocytosis and were classified as $ISMs^-$, whereas the other 3 c-MCAD and all nc-MCAD patients did not. In addition, although both patients with $ISMs^-$ and patients with nc-MCAD presented with idiopathic and allergen-induced anaphylaxis, the former showed a higher frequency of men, cardiovascular symptoms, and insect bite as a trigger, together with greater sBt. Based on a multivariate analysis, a highly efficient model to predict clonality before BM sampling was built that includes male sex ($P = .01$), presyncopal and/or syncopal episodes ($P = .009$) in the absence of urticaria and angioedema ($P = .003$), and sBt >25 μg/L ($P = .006$) as independent predictive factors.

Conclusions: Patients with c-MCAD and $ISMs^-$ display unique clinical and laboratory features different from nc-MCAD patients. A significant percentage of c-MCAD patients can be considered as true $ISMs^-$ diagnosed at early phases of the disease. (J Allergy Clin Immunol 2010;125:1269-78.)

Key words: *Mast cell, mastocytosis, systemic mast cell activation disorders, anaphylaxis, clonal, CD25, KIT mutation, score*

A wide variety of stimuli can trigger activation of mast cells (MCs) in allergic and nonallergic diseases. Cross-linking of FcεRI elicits the release of inflammatory mediators from MC secretory granules[1-3] in IgE-sensitized MCs. Moreover, release of MC mediators can also be elicited through Fcγ receptors,[4-6] complement proteins,[1-3] or aggregated IgG and C3a.[7] Furthermore, MCs express Toll-like receptors 4 and 2, CD48, and complement receptor 1,[8-11] which can activate MCs without requirements for antibody or other immunologic signalling.[12] Disorders characterized by an abnormal MC activation (MCADs) without clear triggers have been described[13,14] and characterized by generalized pruritus, hives, flushing, tachycardia, abdominal pain, diarrhea, and/or syncopal or near-syncopal episodes. Elevated serum baseline tryptase (sBt) in patients with MCAD without evidence for systemic mastocytosis (SM) according to the World Health Organization (WHO) have been reported in some patients with recurrent MCAD.[15]

From [a]Centro de Estudios de Mastocitosis de Castilla La Mancha, Hospital Virgen del Valle, Toledo; [b]the Allergy Unit, Hospital de Fuenlabrada, Madrid; [c]the Allergy Department, Hospital General de Segovia; [d]the Allergy Department, Hospital de Guadalajara; [e]the Allergy Department, Hospital General de Ciudad Real; [f]the Allergy Unit, Fundación Hospital Alcorcón, Madrid; [g]the Allergy Department, Hospital Ramón y Cajal, Madrid; [h]the Allergy Department, Complejo Hospitalario San Millán-San Pedro de La Rioja, Logroño; [i]the Allergy Department, Hospital La Paz, Madrid; [j]the Allergy Department, Hospital Príncipe de Asturias de Alcalá de Henares, Madrid; [k]Centro de Investigación del Cáncer/IBMCC (USAL/CSIC), Departamento de Medicina and Servicio General de Citometría, University of Salamanca, Salamanca; [l]the Pathology Department, Hospital Virgen de la Salud, Toledo; and [m]Red Española de Mastocitosis.

*These authors have contributed equally to this work.

†These authors have contributed equally to this work.

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Reprint requests: Luis Escribano, MD, PhD, Hospital Virgen del Valle, Centro de Estudios de Mastocitosis de Castilla La Mancha, Planta 3ª, Carretera de Cobisa s/n, 45071 Toledo, Spain. E-mail: lescribanom@sescam.jccm.es.

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Abbreviations used
BM: Bone marrow
c-MCAD: Clonal mast cell activation disorder
ISM: Indolent systemic mastocytosis
ISMs$^-$: Indolent systemic mastocytosis without skin lesions
ISMs$^+$: Indolent systemic mastocytosis with skin lesions
MC: Mast cell
MCAD: Mast cell activation disorder
nc-MCAD: Nonclonal mast cell activation disorder
NSAIDs: Nonsteroidal anti-inflammatory drugs
REMA: Spanish Network on Mastocytosis
sBt: Serum baseline tryptase
SM: Systemic mastocytosis
WHO: World Health Organization

Anaphylactic episodes are common in adults with SM (with or without skin involvement) and can be found at frequencies ranging from 22%[16] to 49%.[17] SM in the absence of skin lesions can be associated with aggressive categories of the disease, but indolent SM (ISM) in the absence of skin involvement (ISMs$^-$) has been recognized since 1991.[18] In recent years, the presence of clonal MCs in a subset of patients with idiopathic recurrent anaphylaxis has been reported[19,20] (see also the Online Repository at www.jacionline.org) and named clonal MCAD (c-MCAD)[21] or monoclonal MC activation syndrome.[22,23] Interestingly, in c-MCAD, a lower incidence of both urticaria and angioedema has been reported during acute MC mediator release episodes.[20,24] More recently, proposals and recommendations for an integrated diagnosis of MCAD and c-MCAD[21] together with a series of MCAD associated with *Hymenoptera* sting anaphylaxis have been reported.[25] However, no study has been reported so far in which the clinicobiological features of patients presenting with systemic MC activation symptoms/anaphylaxis in the absence of skin mastocytosis have been prospectively analyzed in patients with clonal versus nonclonal MCs.

Here we describe the clinical, biological, and molecular characteristics of adult patients with symptoms suggesting a MC disorder in the absence of skin lesions with special emphasis on the differences found between patients with c-MCAD fulfilling criteria for ISM (ISMs$^-$) versus nonclonal MCAD (nc-MCAD); in addition, we also investigate the differences between patients with ISMs$^-$ and typical ISM patients with skin lesions (ISMs$^+$). Our results indicate that patients with clonal disorders display unique clinical and laboratory features, and the vast majority correspond to ISMs$^-$ patients whose diagnosis is made at an early phase of the disease with lower MC burden than ISMs$^+$.

METHODS

Patient groups

Adult patients referred to the Instituto de Estudios de Mastocitosis de Castilla La Mancha—the Clinical Reference Centre for the Spanish Network on Mastocytosis (REMA)—with severe and systemic symptoms attributable to MC mediators release episodes/anaphylaxis in the absence of mastocytosis-associated skin lesions, who gave their informed consent to participate in the study according to the local Ethics Committees (n = 91), were selected. Symptoms such as syncope, hypotension, cardiac arrest, dyspnea, abdominal cramping, diarrhea, generalized flushing, urticaria, angioedema, and headache, among others, were recorded. In addition, a control group of 114 patients with ISMs$^+$ (28 of them with anaphylactic episodes) was analyzed.

A complete clinical work-up including careful cutaneous examination to rule out the presence of skin mastocytosis was systematically performed. Peripheral blood cell counts and differential, routine blood biochemistry, sBt (CAP; Phadia Diagnostics, Uppsala, Sweden), total serum IgE, and vitamin B12 were studied in all patients. After a careful clinical work-up was performed and the suspected triggers were identified (eg, *Hymenoptera* sting, drugs, or food, among others) a complete allergy study was performed, including specific IgE (Immuno CAP; Phadia Diagnostics) and skin tests—skin prick test and/or intradermoreaction—as previously described.[16] Skeletal x-ray survey and abdominal ultrasonography or computed tomography scan were performed in all patients, whereas dual energy x-ray absorptiometry was performed in 62 patients. The presence of osteoporosis was defined following well established criteria[26-28]; the presence of bone sclerosis, as assessed by skeletal x-ray survey and/or computed tomography scan, was also recorded.

Morphologic, immunophenotypical, and molecular studies of bone marrow mast cells

In all patients, a complete bone marrow (BM) study was performed strictly following recently proposed criteria.[23] BM biopsy was performed at the outpatient consultation, and patients were premedicated with dexchlorpheniramine, ranitidine, and diazepam 1 hour before the procedure; in 1 patient, BM biopsy was performed at the intensive care unit because of a previous history of stress-induced anaphylaxis; BM MC morphology was analyzed in toluidine blue and May-Grünwald-Giemsa stained smears[29]; BM sections stained for hematoxylin-eosin, Giemsa, reticulin, tryptase, and c-kit were analyzed.[30,31] For both cytologic and histologic studies, all samples were analyzed and reviewed by 3 independent experts (L.E., I.A.-T., M.M.).

Immunophenotypical analysis of CD25 expression on BM MCs was performed by flow cytometry using a multiparameter 4-color immunofluorescence technique according to consensus procedures and criteria previously defined by the REMA.[32,33] Presence of a double MC population (CD25$^+$ and CD25$^-$ MC) was defined when >1% CD25$^-$ MCs were detected within the overall BM MC compartment. In addition, 70 of 83 BM samples were studied in parallel in 2 different laboratories of the REMA for external quality control purposes.

Detection of somatic activating codon Asp816→Val *KIT* mutation or other *KIT* mutations was performed in genomic DNA from fluorescence-activated cell sorting–purified populations of BM MCs, neutrophils, eosinophils, monocytes, lymphocytes, CD34$^+$ hematopoietic progenitor and precursor cells, and nucleated red cells, as described elsewhere.[34,35] To evaluate clonality, the pattern of inactivation of chromosome X was studied by the HUMARA assay[36] in 2 women lacking *KIT* mutations.

Definitions

According to previous reports,[13,15,37] MCAD was defined as the presence of clinical symptoms attributable to MC mediators release independently of biological demonstration of MC activation through, for example, increased sBt levels. All 83 patients studied fulfilled these criteria.

Clonality was defined on the basis of the presence of both *KIT*-mutated MCs (or a clonal HUMARA test in women lacking *KIT* mutation) and aberrant CD25 expression on BM MCs. Those patients lacking both criteria were considered nonclonal. Patients having CD25$^{bright+}$ expression with negative (n = 1) or not evaluable (n = 4) *KIT* mutation, or those carrying *KIT* mutation with a normal MC immunophenotype (n = 3), even if they had elevated sBt, were excluded from the study. According to such criteria, 51 patients were subclassified as having ISMs$^-$ or other c-MCAD and 32 as having nc-MCAD, and 8 were excluded from the study. All ISMs$^+$ showed CD25$^{bright+}$expression in association with *KIT* mutation.

Anaphylaxis was defined following previous published criteria[38,39]; accordingly, those patients with symptoms involving ≥2 organs and those having severe cardiovascular involvement—for example, reduced blood pressure, syncope, and/or cardiac arrest (even in the absence of other organ-related symptoms)—were considered to have anaphylaxis. Both the clinical characteristics of acute MC mediators release episodes and MC-related symptoms in between them were recorded (Table I).

TABLE I. Clinical and laboratory features of patients in between the acute episodes

	nc-MCAD (n = 32)	*P* value	ISMs$^-$ (n = 48)	*P* value	ISMs$^+$ (n = 114)
Male	10/32 (31)	<.001	35/48 (73)	.001	52/114 (46)
Age at onset*	39 (15-65)	NS	41 (18-76)	<.001	28 (0-66)
Age at diagnosis†	53 (19-70)	NS	48 (18-77)	.01	41 (21-79)
Follow-up from onset‡	86 (13-386)	NS	67 (8-332)	<.001	158 (42-497)
Follow-up from diagnosis‡	12 (2-53)	.01	22 (2-95)	.01	40 (2-148)
Pruritus	11/32 (34)	NS	12/48 (25)	<.001	89/112 (79)
Flushing	10/32 (31)	NS	14/48 (29)	.01	57/112 (51)
GI symptoms	5/32 (16)	NS	9/48 (19)	.01	43/112 (38)
Neuropsychiatric§	2/32 (6)	NS	2/48 (4)	NS	8/112 (7)
Organomegaly‖	0/32 (0)	NS	5/48 (10)	NS	19/114 (17)
Osteoporosis¶	2/20 (10)	NS	10/42 (24)	NS	16/102 (16)
Diffuse bone sclerosis#	0/32 (0)	NS	2/48 (4)	NS	10/114 (9)
Total IgE (KU/L)	62.9 (1-553)	NS	33.2 (5-596)	NS	28 (2.3-195)††
Specific IgE	12/27 (44)	NS	25/44 (57)	NS	6/12 (50)††
Tryptase (μg/L)	15.4 (2.7-30)	<.001	25.2 (6.8-515)	NS	40.2 (5.6-644)
Eosinophils >0.5 × 10^9/L	0/32 (0)	NS	2/45 (4)	NS	5/113 (4)
Cytopenia**	1/32 (3)	NS	3/48 (6)	NS	3/114 (3)
Vitamin B12 >1500 pg/mL	0/27 (0)	NS	0/43 (0)	NS	7/103 (7)
Cholesterol <120 mg/dL	1/32 (3)	NS	0/47 (0)	NS	4/112 (4)
Triglycerides <40 mg/dL	2/32 (6)	NS	0/45 (0)	NS	4/112 (4)

Results expressed as number of patients/total patients studied (percentage) for categorical variables and median (range) for continuous variables. Both clinical and laboratory data included in the table correspond to the first study available in each patient.

GI, Gastrointestinal, *NS*, not statistically significant.

*Date of the first MC mediators release episode or appearance of skin lesions.

†Date of BM study.

‡In months.

§Irritability, lost of concentration, severe sleep disturbances.

‖As assessed by computed tomography scan and/or abdominal ultrasonography.

¶As assessed by dual-energy x-ray absorptiometry following WHO guidelines.

#As assessed by radiograph and/or computed tomography scan.

**Hemoglobin <10 g/dL and/or leukocytes <1 × 10^9/L and/or platelets <100 × 10^9/L.

††In the subset of patients with ISM with anaphylaxis (n = 28).

Statistical methods

For all continuous variables, median and range were calculated, whereas for categorical variables, frequencies were reported. The Mann-Whitney *U* and the χ^2 tests were used to assess the statistical significance of differences observed between groups for continuous and categorical variables, respectively. To identify the best combination of independent factors associated with each subgroup of patients, a multivariate (logistic regression) analysis was performed. For multivariate analyses, only those variables that showed statistically significant differences in the univariate study were selected. *P* values ≤.05 were considered to be associated with statistical significance. For all statistical analyses, the SPSS 15.0 statistical software package (SPSS, Chicago, Ill) was used.

RESULTS

Diagnostic criteria for systemic mastocytosis

A total of 83 adults, 37 women (45%) and 46 men (55%) with a median age of 49 years (range, 18-77 years) who were referred to the REMA from April 2001 to January 2009, were studied. After careful analysis of BM samples, 51 (61%) patients were found to have both CD25$^{bright+}$ MCs and either *KIT* mutation (n = 50; the D816V *KIT* mutation in 47 patients and the D816Y, D816H, and Ins815-816 *KIT* mutational changes in 1 patient each) or a clonal HUMARA test (n = 1), and they were categorized as c-MCAD. The remaining 32 patients (39%) showed a normal MC immunophenotype and neither *KIT* mutation nor clonal MCs, and they were classified as having nc-MCAD.

Except for increased sBt, no other major or minor criteria for SM were found in nc-MCAD. In contrast, 48 of 51 clonal patients (94%) fulfilled either 1 major and ≥1 minor (67%) or ≥3 minor criteria (33%) for SM, and they were classified as ISMs$^-$; conversely, another 3 patients who fulfilled only 2 minor criteria (CD25$^{bright+}$ and *KIT* mutation) could not be classified as having SM and were categorized as having c-MCAD. All patients with ISMs$^+$ fulfilled the WHO criteria for SM. MC aggregates were found in 80% of patients. Interestingly, ISMs$^-$ patients showed a higher frequency of lymphoid aggregates ($P = 005$), BM fibrosis ($P = .006$), and lesions constituted by an admixture of MCs, eosinophils, and lymphocytes ($P < .001$) and greater percentages of BM MCs ($P < .001$) versus nc-MCAD (Table II). Furthermore, no differences regarding BM MC percentages were found between nc-MCAD (median, 0.007; range, <0.0001-0.2) and those 3 patients of c-MCAD (median, 0.002; range, 0.0007-0.03) that did not fulfill the WHO criteria for SM. In turn, the only significant differences between ISMs$^-$ and ISMs$^+$ patients were a lower frequency of BM fibrosis ($P = .02$) and a higher frequency of patients showing coexistence of normal CD25$^-$ and aberrant CD25$^+$ BM MCs (double MC population) as assessed by flow cytometry ($P = .02$), among the former group (Table II); similarly, no significant differences were detected between ISMs$^-$ and the other 3 clonal MCAD patients not fulfilling the criteria for SM. Of note, the frequency of *KIT* mutations involving hematopoietic cell lineages other than MC was significantly higher among ISMs$^+$ versus ISMs$^-$ (22% vs 6%; $P = .01$; Table II).

Overall, patients with ISMs$^-$ showed similar clinical and laboratory features to those of patients with nc-MCAD except for a higher frequency of men ($P < .001$) and increased sBt

TABLE II. Morphologic, immunophenotypical, and molecular characteristics of patients

	nc-MCAD n = 32	*P* value	ISMs$^-$n = 48	*P* value	ISMs$^+$ n = 114
BM MC aggregates†	0/31 (0)	<.001	30/45 (67)	NS	90/112 (80)
Hypercellularity†	0/31 (0)	NS	5/44 (11)	NS	21/110 (19)
Lymphoid aggregates†	3/31 (10)	.005	17/44 (39)	NS	57/109 (52)
BM fibrosis†	1/31 (3)	.006	12/43 (28)	.02	53/110 (48)
BM sclerosis†	0/31 (0)	NS	2/43 (5)	NS	15/109 (14)
Abnormal BM MCs‡	0/31 (0)	<.001	46/46 (100)	NS	107/111 (96)
Myelodysplasia‡	2/31 (6)	NS	3/45 (7)	NS	4/111 (4)
BM eosinophilia‡	10/31 (32)	NS	22/45 (49)	NS	45/112 (40)
MEL lesions‡	0/30 (0)	<.001	23/45 (51)	NS	64/111 (58)
Aberrant BM MC§ phenotype§	0/32 (0)	<.001	48/48 (100)	NS	114/114 (100)
Double BM MC population§‖	0/32 (0)	<.001	16/48 (33)	.02	20/113 (18)
BM MCs (%)§#	0.007 (0-0.2)	<.001	0.09 (0.0006-0.5)	NS	0.1 (0.003-1.47)
Positive *KIT* mutation	0/32 (0)	<.001	47/48 (98)	NS	114/114 (100)
Multilineal *KIT* mutation¶	0/32 (0)	<.001	3/47 (6)	.01	24/106 (23)

Results expressed as number of patients/total patients studied (percentage) or as #median (range). Clinical and laboratory data included in the table correspond to the first study available in each patient. *MEL,* Lesions constituted by an admixture of MCs, eosinophils, and lymphocytes.

†In tryptase-stained BM sections.

‡In May-Grünwald-Giemsa-stained BM smears.

§As assessed by flow cytometry.

‖Coexistence of immunophenotypically normal (CD25$^-$) and aberrant (CD25$^+$) BM MCs.

¶Detection of *KIT* mutation involving BM MCs and other BM cell lineages.

($P < .001$) (Table I). In turn, differences between patients with ISMs$^-$ and ISMs$^+$ included male predominance ($P = .001$), older age at both disease onset ($P < .001$) and diagnosis ($P = .01$), and a lower frequency of pruritus ($P < .001$), flushing ($P = .01$), and gastrointestinal symptoms ($P = .01$) among the former group (Table I).

Clinical findings

After a median follow-up of 71 months (range, 8-386 months), a total of 162 acute MC mediators release episodes were recorded in 70 patients, whereas the remaining 13 patients had a countless number of episodes, with no significant differences between ISMs$^-$ and nc-MCAD patients (Fig 1, *A*). Overall, the most common triggers for acute episodes of MC mediators release were insects (n = 44; 53%), including wasp (n = 30), bee (n = 10), both wasp and bee (n = 1), horsefly (*Hippobosca equina*; n = 1), and unidentified insects (n = 2). In 36 of 83 (43%) patients (88% of the 41 cases triggered by *Hymenoptera* sting), *Hymenoptera* sting was the only trigger identified: 27 corresponded to ISMs$^-$ and 2 to c-MCAD, and 7 were nc-MCAD patients. Drug reactions were found in 16 patients (19%), 5 with ISMs$^-$ and 11 with nc-MCAD, to antibiotics (n = 6)—amoxicillin (n = 3), penicillin (n = 1), streptomycin and teicoplanin (n = 1), and fosfomycin (n = 1)—and nonsteroidal anti-inflammatory drugs (NSAIDs) in 5—metamizole (n = 2), aspirin (n = 1), aspirin and naproxen (n = 1), and ibuprofen (n = 1). Two patients experienced episodes triggered by both NSAIDs and betalactamics, and the remaining 3 drug-induced cases included reactions triggered by codeine (n = 1), mepivacaine (n = 1), and rocuronium (n = 1). Among the drug reactions, 15 were immediate (≤2 hours after contact with the trigger), and 1 caused by NSAIDs was delayed 8 hours after contact with the trigger. Other triggers were fish (n = 4), peach (n = 1), egg and sunflower oil (n = 1), orange candy (n = 1), alcohol (n = 1), and intestinal manipulation during abdominal surgery (n = 1). Twenty-nine patients (35%) were classified as idiopathic because no identifiable cause was found. In 24 of 33 patients (73%) with a single MC mediators release episode, this was caused by insects, whereas those patients having >5 episodes (n = 17) were mainly idiopathic (53%) or triggered by mixed causes (47%); in 16 of 50 patients (32%) with ≥2 episodes, each episode was caused by a different trigger.

According to the final diagnosis (Fig 1, *B*), insects were the most common trigger among patients with ISMs$^-$ (65% vs 31% in nc-MCAD; $P = .003$), whereas drug-induced MC mediators release was characteristic of the nc-MCAD group (34% vs 10% among ISMs$^-$ patients; $P = .009$), with almost half of these latter patients showing idiopathic MC mediators release. Among the 3 patients of c-MCAD, 2 were triggered only by insects and 1 by both insects and fish.

Sixty-six patients (80%), 21 of them corresponding to nc-MCAD, had life-threatening cardiovascular and/or respiratory symptoms, whereas 17 (20%) presented with other severe MC mediator–related symptoms. Regarding the clinical symptoms of the most severe episode, significantly higher frequencies of skin (urticaria and/or angioedema; $P < .001$) and respiratory (dyspnea; $P = .005$) symptoms were found among nc-MCAD versus ISMs$^-$, whereas cardiovascular (presyncope, $P = .001$; syncope, $P = .001$) symptoms were more frequently found among the latter group (Fig 2, Fig E1); in contrast, similar frequencies were detected for each individual symptom between patients with ISMs$^-$ and ISMs$^+$ with anaphylactic reactions (data not shown). Interestingly, no differences were found among nc-MCAD patients regarding severity of acute episodes between patients displaying increased (n = 23) versus normal (n = 9) sBt: respiratory compromise (74% vs 67%), presyncope (35% vs 56%), syncope (39% vs 44%), hypotension (30% vs 33%), and cardiac arrest (0% vs 11%), respectively. In addition, when comparing ISMs$^-$ patients with *Hymenoptera* sting as the only trigger (n = 27) with other ISMs$^-$ patients (n = 21), a greater predominance in men ($P = .005$), together with a lower frequency of recurrent acute episodes ($P < .001$) and both flushing and gastrointestinal symptoms during acute episodes ($P < .001$) as well as in between them ($P = .006$), was found among the former group; in contrast,

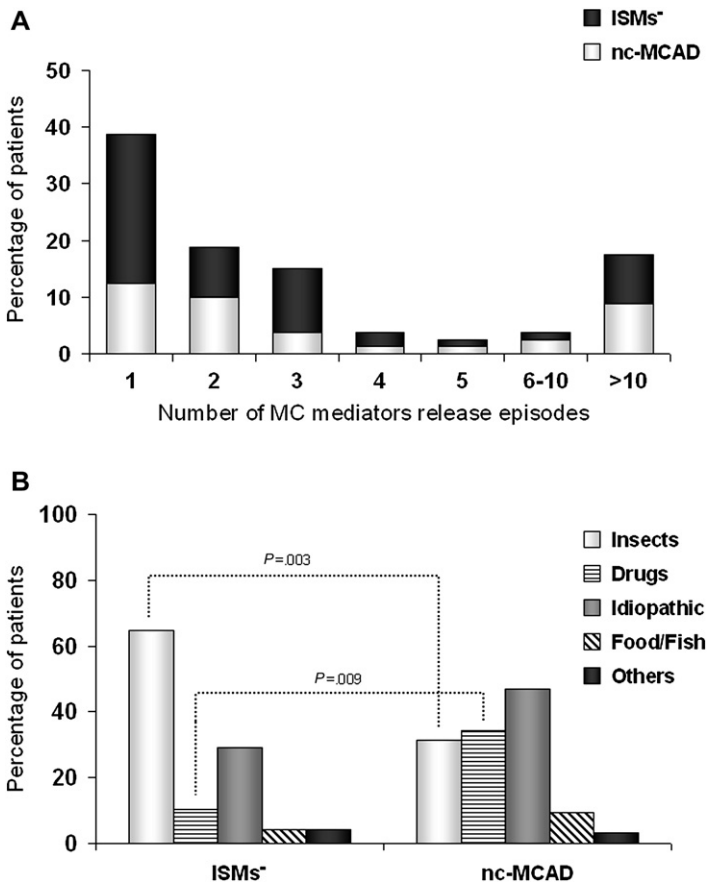

FIG 1. Distribution of nc-MCAD and ISMs$^-$ patients (n = 80) according to the number of acute episodes after a median follow-up before the referral of 71 months (8-386 months; **A**) and the frequency of different triggers for the release of MC mediators **(B)** in ISMs$^-$ (n = 48) vs nc-MCAD (n = 32). No statistically significant differences regarding the median follow-up were found between clonal and nonclonal patients. In contrast, the median (range) follow-up of patients with a single MC mediators release episode was significantly lower ($P < .001$) than that of patients with >1 episode: 44 months (8-380 months) vs 103 months (13-386 months).

no differences ($P > .05$) regarding bone loss were found (61% vs 63%, respectively).

Overall, anesthetic procedures were carried out in 25 patients; anaphylactic episodes developed in 3 patients, including 2 of 13 nc-MCAD (triggered by rocuronium and mepivacaine, respectively), 1 of 11 patients with ISMs$^-$ who had a cardiac arrest related to gastrointestinal manipulation during a laparotomy, and 0 of 1 c-MCAD.

Interestingly, 4 of 28 (14%) patients with ISMs$^+$ with anaphylactic reactions had MC mediators release episodes at 12, 14, 19, and 32 months before the development of skin lesions and diagnosis of ISM. Three of them were idiopathic, and 1 was a result of an IgE-mediated amoxicillin reaction.

Serum tryptase levels

Median sBt was significantly higher ($P < .001$) among patients with ISMs$^-$ versus nc-MCAD. In line with this, sBt <15 μg/L and <11.5μg/L were found in only a minority of all clonal patients—5 (10%) and 2 (4%), respectively—but in a significant proportion of all patients with nc-MCAD—15 (47%) and 9 (28%), respectively ($P < .001$ and $P = .005$, respectively), whereas sBt >25 μg/L was seen in 24 (50%) and 4 (12%) ISMs$^-$ and nc-MCAD patients, respectively ($P = .001$). An increased frequency of ISMs$^-$ versus nc-MCAD was found among patients with sBt levels >20 μg/L—35 (78%) versus 10 (22%), respectively—and a lower incidence of ISMs$^-$ versus nc-MCAD among patients with sBt levels <20 μg/L—13 (37%) versus 22 (63%), respectively ($P < .001$). No significant differences were found in sBt levels of patients with ISMs$^-$ showing *Hymenoptera* sting as the only trigger versus other ISMs$^-$ patients. The 3 c-MCAD patients showed sBt levels of 9.5 μg/L, 12.8 μg /L, and 14.3 μg/L, respectively.

Allergy study

A complete allergic work-up was made in 74 patients (CAP, n = 24; skin test, n = 1, both, n = 49). In 40 patients (54%), an underlying IgE-mediated mechanism responsible for acute episodes was demonstrated, with no significant differences ($P > .05$) between patients with ISMs$^-$ and nc-MCAD (57% vs 44% of positive tests, respectively). In addition, an IgE-mediated mechanism was detected in all 3 c-MCAD patients (two patients with hymenoptera sting anaphylaxis and one patient with hymenoptera sting anaphylaxis associated with fish allergy).

Specific IgE was found in 35 of 41 (85%) cases triggered by *Hymenoptera* sting, mostly corresponding to ISMs$^-$ (n = 23; 66%)

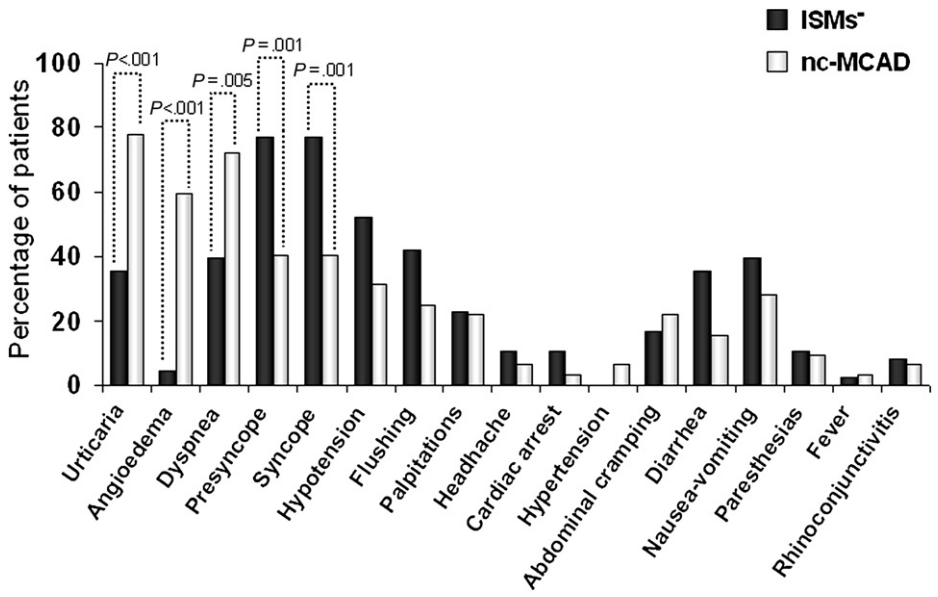

FIG 2. Overall distribution of clinical symptoms associated with the most severe MC mediators release episodes in ISMs$^-$ (n = 48) vs nc-MCAD patients (n = 32).

and other clonal MCAD patients not fulfilling the criteria for SM (n = 3; 9%); in all 6 patients lacking specific IgE antibodies against *Hymenoptera* venom, the diagnosis of ISMs$^-$ was confirmed. Besides acute episodes, associated allergic diseases were also diagnosed in 7 ISMs$^-$ (1 contact dermatitis, 5 rhinoconjunctivitis, and 1 asthma) and in 5 nc-MCAD patients (2 rhinoconjunctivitis, 1 rhinitis, and 2 urticaria—1 because of grass pollen and 1 because of penicillin).

Predictive model for MC clonality (ISMs$^-$ and other c-MCADs vs nc-MCAD) based on clinical and laboratory findings

Tables III and IV show the results of both the univariate and multivariate analyses of predictive factors for the identification of clonal patients (ISMs$^-$ and c-MCAD; Table III) versus nc-MCAD (Table IV). Based on those variables with independent predictive value in the multivariate analysis, a scoring model was built to predict MC clonality (Fig 3) with a sensitivity and a specificity of 92% (95% CI, 85% to 100%) and 81% (95% CI, 68% to 95%), respectively, a positive predictive value of 89% (95% CI, 80% to 97%), and a negative predictive value of 87% (95% CI, 75% to 99%). Interestingly, when the same score was specifically applied to those 36 patients with *Hymenoptera* sting as the only trigger, the sensitivity increased up to 97% (95% CI, 90% to 100%) with a specificity of 71% (95% CI, 38% to 100%) versus 86% (95% CI, 72% to 100%) and 84% (95% CI, 70% to 98%) when applied to the remaining 47 patients, respectively.

DISCUSSION

Here we report on the largest cohort of patients presenting with severe MC mediator symptoms/anaphylaxis, in the absence of mastocytosis of the skin, in which detailed clinicobiological, morphologic, and molecular analyses were prospectively performed, aimed at discriminating between clonal and nonclonal patients. Because all patients shared symptoms related with systemic MC mediators release, the term *systemic* MCAD might be more appropriate than just MCAD for clear distinction from other allergic and nonallergic diseases (eg, asthma, rhinitis, conjunctivitis, hypersensitivity diseases, or irritable bowel disease). Inclusion criteria required concordant immunophenotypic and molecular findings, and only a small proportion (<10%) of all consecutive patients analyzed had to be excluded because of lack of clonal molecular markers in the presence of immunophenotypically aberrant BM MCs.

In our series, based on the clonal nature of BM MCs, 2 different molecular subgroups of patients were defined: clonal and non-clonal patients. The vast majority of the clonal patients fulfilled the WHO diagnostic criteria for SM[23,40,41] and thus, they were classified as ISMs$^-$; the median follow-up for this group was of 22 months (range, 2-95 months); only 3 clonal patients did not fulfill the criteria for SM at the moment they entered the study. However, WHO diagnostic criteria for SM (3 minor criteria) were fulfilled in 1 of them 26 months after the referral because of a sustained increase of sBt >20 μg/L; the other 2 patients had a shorter follow-up of 8 and 11 months. Altogether, these results would support the notion that a strict follow-up should be made for years in patients with c-MCAD who initially do not fulfill the criteria for mastocytosis to rule out the diagnosis of SM. In line with data reported in patients with clonal MCs with *Hymenoptera* sting anaphylaxis in the absence of skin mastocytosis,[25] in our series, two thirds of these patients fulfilled the major criterion (compact clusters of >15 BM MCs), whereas in the remaining patients, either 3 or 4 minor criteria were fulfilled. Interestingly, ISMs$^-$ showed a lower frequency of BM fibrosis together with a greater frequency of patients in which normal and aberrant MCs coexisted in the BM versus ISMs$^+$ patients. It should be emphasized that ISMs$^-$ clearly differs from the so-called isolated BM mastocytosis, an exceptional subcategory of SM in the absence of both skin lesions and MC mediator–related symptoms with a low MC burden[40,41] whose diagnosis is

TABLE III. Univariate and multivariate analysis of clinical and laboratory variables associated with c-MCAD

	c-MCAD/ISMs$^-$				
	Univariate analysis			Multivariate analysis	
Variable	Patients (%)	RR (95% CI)	*P* value	HR (95% CI)	*P* value
Male	70.6	5.28 (2.02-13.78)	.001	4.77 (1.38-16.4)	.013
Absence of urticaria and angioedema*	62.7	9.09 (2.99-27.6)	<.001	5.39 (1.50-19.3)	.003
Dizziness and/or syncope*	94.1	9.60 (2.44-37.7)	.001	14.6 (1.94-109.8)	.009
Insect as trigger	66.7	4.4 (1.70-11.3)	.002		
Serum tryptase >25 μg/L†	47.1	6.22 (1.90-20.3)	.002	10.4 (1.99-54.7)	.006

Only those variables showing statistically significant differences in the univariate analysis for c-MCAD/ISMs are shown and were used in the multivariate analysis.
HR, Hazard ratio; *RR*, relative risk.
*During the most severe MC mediators release episode.
†Adjusted median of baseline serum tryptase at diagnosis in c-MCAD/ISMs$^-$ group.

TABLE IV. Univariate and multivariate analysis of clinical and laboratory variables associated with nc-MCAD

	nc-MCAD				
	Univariate analysis			Multivariate analysis	
Variable	Patients (%)	RR (95% CI)	*P* value	HR (95% CI)	*P* value
Female	68.8	5.28 (2.02-13.7)	.001	3.81 (1.21-12.0)	.022
Urticaria or angioedema*	84.4	9.09 (2.99-27.6)	<.001	7.70 (2.19-26.9)	.001
Respiratory symptoms*	71.9	3.65 (1.41-9.45)	.008		
Drugs as trigger	34.4	4.81 (1.48-15.6)	.009		
Serum tryptase <15 μg/L†	46.9	4.74 (1.70-13.2)	.003	4.77 (1.35-16.8)	.015

Only those variables showing statistically significant differences in the univariate analysis for nc-MCAD are shown and were used in the multivariate analysis.
HR, Hazard ratio; *RR*, relative risk.
*During the most severe MC mediators release episode.
†Adjusted median of baseline serum tryptase at diagnosis in nc-MCAD group.

occasionally made during a BM study for pathological conditions other than mastocytosis.

Among patients with ISMs$^-$, our results show an increased male predominance, as also described for ISMs$^+$ patients with anaphylaxis.[16] Furthermore, a higher frequency in males has been recently described among patients with ISM affected by IgE-mediated anaphylaxis versus adult patients without SM (2.3:1[16] vs 1:1[42]). Altogether, these results suggest the existence of a relationship between male sex and the severity of symptoms associated with the release of MC mediators in mastocytosis, especially among ISMs$^-$. Of note, in this study, male predominance was particularly high among patients with ISMs$^-$ with *Hymenoptera* sting as the only trigger, with its frequency rising up to 6.1:1.

In addition, our results show an association between the presence of anaphylaxis with cardiovascular symptoms in the absence of both urticaria and angioedema and ISMs$^-$, in contrast with patients who did not show clonal MC in their bone marrow. These findings confirm and extend previous observations in smaller series of patients[20,24] and indicate that mastocytosis should be suspected in patients with recurrent anaphylaxis lacking skin mastocytosis who present with syncopal or near-syncopal episodes[22,43] without associated hives or angioedema.[44] Such differences between ISMs$^-$ and nc-MCAD patients regarding MC mediator–related symptoms during acute episodes cannot be directly attributable to the lower MC numbers observed in nc-MCAD because no differences were found in this regard in nc-MCAD patients with or without cardiovascular symptoms or between nc-MCAD and clonal MCAD patients not fulfilling the criteria for SM; alternatively, differences in tissue distribution of MC from clonal versus nonclonal patients and/or the MC activation and/or inhibition pathways involved in both patient groups could contribute to explaining their different clinical behavior.

The WHO recommends the use of increased sBt levels of >20 μg/L as a minor diagnostic criterion for SM.[23,40,41] However, we found sBt levels >20 μg/L in around 20% to 25% of all patients of nc-MCAD, and Kassab et al[15] reported 4 of 48 patients with recurrent unexplained MC activation symptoms without evidence for cutaneous or SM; in turn, 4% of all our ISMs$^-$ and 6% of all clonal patients (including both ISMs$^-$ and other c-MCAD) showed normal sBt levels (<11.5 μg/L). In fact, we show that a higher sensitivity and specificity to predict for clonality in patients without skin mastocytosis could be achieved once sBt values >25 μg/L or <15 μg/L were used in combination with other parameters; conversely, a mild increase in sBt (between 15 μg/L and 25 μg/L) alone had a limited predictive value for clonality in such patients. Furthermore, no clear relationship was found between sBt levels and the severity of clinical symptoms in ISMs$^-$, c-MCAD, and nc-MCAD, supporting the notion that sBt by itself cannot be considered a predictor for the severity of symptoms.

As previously reported,[16,25] our results also show that the most common trigger for anaphylactic episodes is *Hymenoptera* sting, especially among clonal patients. In contrast, drugs were mostly involved as a trigger in nc-MCAD. Interestingly, the relative distribution of triggers among patients with nc-MCAD (but not ISMs$^-$ or other clonal patients) showed a similar pattern to that of patients with ISMs$^+$ with anaphylactic episodes (data not shown). Nevertheless, because Instituto de Estudios de Mastocitosis de Castilla La Mancha: Clinical Reference Center of the Spanish Network on Mastocytosis is a reference center for

VARIABLE		SCORE
GENDER	Male	+1
	Female	-1
CLINICAL SYMPTOMS	Absence of urticaria and angioedema	+1
	Urticaria and/or angioedema	-2
	Presyncope and/or syncope	+3
TRYPTASE*	<15 ng/mL	-1
	>25 ng/mL	+2

*Baseline serum tryptase

SCORE < 2: low probability of clonal MCAD

SCORE ≥ 2: high probability of clonal MCAD

Sensitivity: 0.92
Positive Predictive Value: 0.89

Specificity: 0.81
Negative Predictive Value: 0.87

FIG 3. Scoring model proposed as a screening tool for the presence of clonal MCs in patients presenting with anaphylaxis in the absence of skin mastocytosis before a BM study.

Hymenoptera sting anaphylaxis, the prevalence of *Hymenoptera* sting as a trigger might be overestimated in our series. Furthermore, most cases triggered by *Hymenoptera* sting having specific IgE antibodies and all patients with a negative allergy study corresponded to ISMs$^-$, indicating that in both circumstances, ISMs$^-$ should be suspected. Despite the fact that ISMs$^-$ patients that were only triggered by *Hymenoptera* venom shared several mastocytosis-associated events with other ISMs$^-$ and ISMs$^+$ patients (eg, the frequency of bone loss), the clinical behavior of the disease in between the acute episodes was characterized by a particularly lower frequency of symptoms (data not shown); the underlying mechanisms responsible for such a low baseline MC activation remain unknown.

Altogether, our findings suggest that diagnosis of ISMs$^-$ and other c-MCAD patients is frequently made in an early phase of the disease with lower MC burden than observed in ISMs$^+$. However, this could be also partially explained by the shorter time between the onset of symptoms and the BM study among both groups versus ISMs$^+$ patients. In line with this hypothesis, periodical monitoring of sBt carried out in all patients as per the REMA protocol showed that in one third of patients with c-MCAD who initially met only the immunophenotypical and molecular WHO diagnostic criteria for SM, 3 minor criteria were fulfilled after 26 months because of a sustained increase of sBt >20 μg/L; in the other 2 patients, sBt remained below 20 μg/L 8 and 11 months after diagnosis. In addition, in 4 of 28 (14%) patients with ISMs$^+$ with anaphylactic reactions, skin lesions appeared 12, 14, 19, and 32 months after the first MC mediators release episode, respectively. In contrast, at the time of closing this study, none of the 48 patients with ISMs$^-$ had developed skin lesions after a median follow-up of 67 months (range, 8-380 months). The higher frequency of *KIT* mutation found among ISMs$^-$ (and the 3 patients with c-MCAD who did not meet the criteria for SM) showing an aberrant BM MC phenotype in our series versus previous reports[19,20,25] could be a result of the investigation of *KIT* mutation in fluorescence-activated cell sorting–purified BM MCs (vs total mononuclear cells) in our study because of the greater sensitivity of the method.[20] We have recently described the association of ISM carrying *KIT* mutation in all myeloid and lymphoid BM cell lineages in addition to MCs, and a higher risk of disease progression, versus ISM with a more restricted involvement of hematopoietic cell lineages and *KIT* mutation limited to MCs.[45] In our patients with ISMs$^-$ as well as the other 3 clonal patients, the *KIT* mutation was restricted to BM MCs in all but 3 ISMs$^-$ patients (involving other myeloid cells and both myeloid and lymphoid cells in 2 patients and 1 patient, respectively), suggesting that long-term prognosis of ISMs$^-$ could be even slightly better than that of ISMs$^+$, at least in terms of disease progression. However, the morbidity rate of ISMs$^-$ is not negligible because of (1) substantial impairment of the quality of life because of the recurrent nature of the episodes in many patients; (2) life-threatening complications occurring in a subset of patients, especially those with cardiovascular collapse or severe respiratory involvement; (3) a higher risk of MC mediators–related symptoms in patients undergoing medical procedures such as anesthesia or receiving contrast media; and (4), the relatively high risk of osteoporosis (even in young male patients) with the subsequent increased risk of bone fractures.

In summary, our results indicate that a significant percentage of clonal patients presenting with systemic MC activation symptoms in the absence of skin mastocytosis correspond to ISM at early phases of the disease typically characterized by (1) the absence of skin lesions, (2) a high prevalence in men, (3) anaphylaxis associated with cardiovascular symptoms in the absence of urticaria or angioedema frequently triggered by *Hymenoptera* sting, (4) fewer MC-related symptoms between the acute episodes associated with lower sBt than in ISMs$^+$, (5) an increased incidence of osteoporosis, (6) a decreased frequency of BM fibrosis,

(7) a higher frequency of patients with coexisting normal and aberrant BM MCs, and (8) *KIT* mutation usually restricted to MCs. Further follow-up of patients fulfilling only 1[46] or 2 minor criteria for SM is warranted to determine whether they evolve to a true SM or whether represent a distinct disease subtype.[44] Similarly, periodical monitoring of the skin should be performed in all ISMs$^-$ patients to check for the potential future development of typical mastocytosis skin lesions. Finally, close follow-up of those few patients carrying multilineal *KIT* mutations must be performed to detect early signs of disease progression.

Clinical implications: A significant percentage of c-MCADs represents a variant of ISM.

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A surprisingly high prevalence of D816V *KIT* mutation has been previously reported in normal subjects (2/9), atopic subjects (2/10), subjects with food anaphylaxis (5/11), subjects with anaphylaxis not triggered by foods (4/10); nevertheless, SM was not ruled out in any of these patients. Thus, caution should be taken in considering such results before their confirmation in independent series.[E1] In this regard, we have performed *KIT* mutational analysis in purified BM MCs from 31 patients without mastocytosis with myelodysplastic disorders (n = 17), acute myeloblastic leukemia (n = 10), and reactive cytopenias (n = 4), and none of them showed MC *KIT* mutation.

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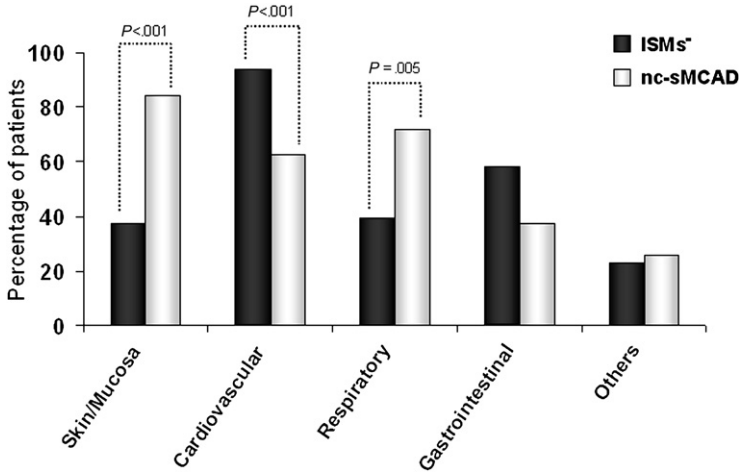

FIG E1. Distribution of clinical symptoms associated with the most severe MC mediators release episodes grouped by organ involvement in ISMs$^-$ (n = 48) vs. nc-MCAD patients (n = 32).